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and Kircher, 2010) were performed in a dedicated ancient DNA laboratory (Paleogenetic Core Facility, ArchaeoBioCenter, LMU Munich, Germany and University of York, UK), following a range of standard contamination precautions. In early-domestic samples the indexed libraries were enriched for camel mtDNA by in-solution hybridization capture (Mycroarray's Mybait kit) and sequenced on a single lane of Illumina HiSeq2000. However, in wild samples an approximately 530 bp fragment of mtDNA control region (Genbank: NC_009849.1, nt 15347 - 15877) were amplified from the indexed ds-DNA libraries, using 10 overlapping primer pairs. In order to confirm the authenticity of the aDNA sequence data, we used the software mapDamage2.0 (Jónsson et al., 2013) to identify these aDNA damage patterns in all mapped sequences.

Results and Discussion

In early-domestic camels, we recovered the two existing modern haplogroups (A and B; Charruau, 2012), which indicate the early and complete presence of both haplogroups in the North Arabian Peninsula from the Early Byzantine onwards. In wild specimens from four different locations in the UAE, three unique haplotypes and four modern haplotypes that clustered with the major haplogroup (B) were detected. This suggests that this region of the UAE is one of the possible locations of camel domestication. The minor haplogroup (A) was not detected in the wild samples collected in this study. This suggests the possible loss of this haplotype during the sampling as a result of its low frequency in the wild dromedary population. Furthermore, we report 14 nearly complete mtDNA from early-domestic dromedaries dated to 1000 – 2000 ybp. Because the environmental conditions of the desert drastically reduce the chances of DNA surviving from poorly preserved specimens, DNA extraction and sequencing remain a challenge. This study highlights one of the few successful recoveries of genetic materials from specimens belonging to hot and arid environments, and reports the first mtDNA recovery from early domestic dromedaries.

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COMBINED SANGER AND NGS SEQUENCE ANALYSIS OF THE MYOSTATIN GENE (MSTN) IN THE CAMELUS DROMEDARIUS SPECIES

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Abstract

Different mutations have been identified in the myostatin gene (MSTN), some of which are responsible for protein inactivation and double muscling phenotype in mammals. So far, no extensive polymorphism survey has been carried out in Camelus dromedarius. We therefore performed a sequence analysis, adopting a combined strategy involving Sanger and next generation sequencing (NGS). Notably, 3.6 kb of the MSTN locus were Sanger sequenced in a population dataset including samples from Algeria (10), Tunisia (5), Egypt (9), Mauritania (5), Sudan (5) and Saudi Arabia (9). A further whole-genome dataset, including 7 C. dromedarius from Pakistan (1), Kenya (1), Saudi Arabia (3), Canary Islands (1) and Oman (1) were sequenced using the Illumina Hi-Seq 2000 technique at an average 15-fold coverage. Whole-genome NGS sequence data from 9 C. bactrianus and 7 C. ferus samples were also available for comparison. Overall, only four polymorphisms were detected, all of them were observed in intronic regions, corresponding to an average presence of one SNP per 1200 bps. Ten fixed sites were observed when comparing C. dromedarius MSTN sequences with those from C. bactrianus and C. ferus. The apparent low sequence diversity observed at the MSTN locus may reflect the peculiar evolutionary history of this species, with purifying selection and drift phenomena as the most likely acting forces.

ДРОМЕДАР ТҮЙЕЛЕРІНІҢ МИОСТАТИН ГЕНІ (MSTN) ТІЗБЕКТЕРІНІҢ КОМБИНИРЛЕНГЕН СЭНГЕР ЖӘНЕ NGS АНАЛИЗІ

Миостатин гені (MSTN) үшін әр түрлі мутациялар идентифицирленді, олардың кейбіреулері ақуыздардың дезактивациясы мен сүтқоректілердің бұлшықеттерінің қос фенотипіне жауап береді. Сол уақыттан бері Camelus dromedarius үшін полиморфизмі туралы ешбір шолу жасалған жоқ. Сондықтан біз Сэнгер стратегиясы мен секвенирлеудің келесі ұрпағын (NGS) тұтастыратын секвенирлеу анализін жүргіздік. Сэнгер әдісі бойынша Алжир

(10), Тунис (5), Египт (9), Мавритания (5), Судан (5) және Сауд Аравиясы (9) популяциялары MSTN локусының 3,6 кб бойынша жасалды. Одан кейін Пакистан (1), Кения (1), Сауд Аравиясы (3), Канар аралы (1) және Оман (1) дромедарларының толық геномы Illumina Hi-Seq 2000 техникасын пайдалана отырып орташа түрде 15 реттік қаптама арқылы секвенирленді. 9 C бактриандардың толық геномды нәтижелері мен NGS секвенирлеу арқылы алынған 7 C. Ferus сынамалары салыстыру үшін алынды. Жалпы түрді тек төрт полиморфизм анықталды, олар орташа түрде 1200 bps сәйкес болатын бір SNP болатындай интронды аймақтарда табылды. C. bactrianus и C. Ferus салыстырғанда он фикстелген орталықтар анықталды. Тізбектердің пайда болған төмен алуантүрлілігі MSTN локусында анықталды, ол бұл түрлердің тазарту селекциясы мен белсенді күш ретінде дрифт феноменінің эволюциялық тарихын көрсете алады.

КОМБИНИРОВАННЫЕ СЭНГЕР И NGS АНАЛИЗЫ ПОСЛЕДОВАТЕЛЬНОСТЕЙ ГЕНА МИОСТАТИНА (MSTN) У ВЕРБЛЮДОВ ДРОМЕДАРОВ

Разные мутации были идентифицированы в гене миостатине (MSTN), некоторые из которых отвечают за дезактивацию белков и двойной фенотип мускулатуры у млекопитающих. С тех пор никакого обширного обзора полиморфизма не было сделано для Camelus dromedarius. Поэтому мы провели анализ секвенирования, включающий в себя стратегию Сэнгер и секвенирование следующего поколения (NGS). Особенно, материал включающий в себя популяции Алжира (10), Туниса (5), Египта (9), Мавритании (5), Судана (5 и Саудовской Аравии (9) были секвенировании по методу Сэнгер с 3,6 кб локуса MSTN. Далее были секвенированы полные данные генома включающие в себя 7 C дромедаров из Пакистана (1), Кении (1), Саудовской Аравии (3), Канарских островов (1) и Омана (1) используя технику Illumina Hi-Seq 2000 в среднем с 15-кратным покрытием. Полные геномные данные 9 C бактрианов полученные с помощью NGS секвенирования и 7 C. ferus образцы были также доступны для сравнения. В целом, было обнаружено только четыре полиморфизма, все они были обнаружены в интронных регионах соответствующих среднему присутствию одного SNP на 1200 bps. Десять фиксированных центров были обнаружены при сравнении C. bactrianus и C. ferus. Появление низкого разнообразия последовательностей было обнаружено в MSTN локусе, что может отражать своеобразную эволюционную историю этих видов с селекцией очистки и феноменами дрифта как действующих сил.

Introduction

Myostatin, also known as Growth Differentiation Factor-8 (GDF-8), is a negative regulator for muscle growth and development that acts suppressing proliferation and differentiation of myoblasts (Thomas *et al.,* 2000). Several functional mutations have been identified in the myostatin (MSTN) gene of different mammalian species, responsible for a “double muscling” phenotype characterized by increase in the number of muscle fibres (hyperplasia) and fibre enlargement (hypertrophy) (McPherron and Lee, 1997). Besides its obvious advantages for the meat industry, the “double muscling” phenotype is also associated with several drawbacks, such as fertility reduction and lower offspring viability (Ménissier, 1982). Other pleiotropic effects associated with MSTN variation have been observed in cattle, where a reduction of total lipid content and connective tissue, high meat yield and meat tenderness have been documented (Ménissier, 1982; Shahin and Berg, 1985; Kobolak *et al*., 2002).

Camels play an important role in arid lands as source of food. The importance of the camel as a meat-producing animal is increasing, also due to the high nutritive value of its meat (Kadim *et al*., 2008). Knowledge about molecular mechanisms modulating muscle mass development in camels are still lacking. We contributed to fill this gap by carrying out a comprehensive characterization of the sequence variability at the MSTN locus in *Camelus dromedarius* integrating NGS and Sanger sequencing data.

Materials and methods

For the Sanger sequencing experiment, *C. dromedarius* blood and muscle samples collected in Algeria (10), Tunisia (5), Egypt (9), Mauritania (5), Sudan (5) and Saudi Arabia (9) were used to isolate genomic DNA by using commercial kits (Qiagen). The three MSTN exons and part of introns were amplified by PCR using heterologous primers designed from the *C. ferus* consensus sequence using Primer3 (www.primer3.ut.ee/, version 4.0.0). PCR products were separated by electrophoresis on a 2% agarose gel. Fragments excised from the gel and purified were Sanger sequenced by the Eurofins MWG Operon service. Sequence analysis was carried out using Bioedit (http://www.mbio.ncsu.edu/bioedit/page2.html). Sequence alignments were performed using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

For the NGS experiment, we extracted DNA from blood samples collected from *C. dromedarius* in Pakistan (1), Kenya (1), Saudi Arabia (3), Sudan (1), Canary Islands (1), UAE (1) and Qatar (1). All samples were sequenced to approximately 15-fold coverage on an Illumina Hi-Seq 2000 using a 500 bp insert paired-end library. All reads were quality trimmed (5’ end quality > 20) and mapped to the *C. ferus* reference genome (Accession GCA_000311805.2), All single nucleotide variants (SNVs) were identified according to the Genome Analysis Toolkit best practices guidelines (Van der Auwera *et al*., 2013). The complete description of methods can be found in Ruiz *et al*. (2015).

Results and discussions

In a previous survey, a very limited region (256 bp) in the first exon of the *Camelus dromedarius* MSTN locus had been screened for sequence polymorphisms (Shah *et al*., 2006). In this study, we report the results of an extensive polymorphisms analysis carried out integrating data on a 3.6 kb region from a traditional Sanger sequencing approach and data on the whole MSTN locus (6.8 kb) from a high-throughput whole-genome NGS approach on a relevant population dataset. Unexpectedly, only four variant nucleotide sites were observed, one transversion (54652_G/C) and three transitions (54964_G/A, 54965_C/T e 56755_T/C), all located in intronic regions. Frequencies of minor alleles were, respectively, 0.21 C; N = 14), 0.42 (G and C; N = 32); 0.14 (C; N = 7). We did not observe any polymorphism in the second exon of the myostatin, which is known from other species to harbour functional mutations (Baron *et al*., 2012). The average SNP density was one SNP every 1712 bps, remarkably lower than reported for the MSTN gene in cattle and sheep (on average, 1 SNP every 100 bp; Dunner *et al*., 2003; Gan *et al*., 2008). Refining our previous hypothesis, we speculate that the low sequence diversity observed at the MSTN locus in *C. dromedarius* may reflects its peculiar evolutionary history.

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SCREENING OF DROMEDARY GENOME WITH HETEROLOGOUS SHORT TANDEM REPEAT (STR) PRIMERS FOR ITS UTILITY IN PARENTAGE TESTING

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Abstract

"Even though many dromedary specific Short Tandem Repeats (STR) loci are available in single humped camels, the need for more informative loci still exists to improve parentage testing in camels. Further, almost all the available camel specific STRs are di-nucleotide loci which are known for their stutter formation. Incorporation of tetra-nucleotide loci can improve parentage testing considerably. With this objective, more than 150 heterologous STR primers from different species including Bactrian camel, alpaca, llama, vicuna, guanaco and pig were tested on the dromedary genome. The screening was done at two stages: first, the primers were tested on a panel of 20 unrelated animals. These primers included both di-nucleotide and tetra-nucleotide STRs. Genetic variability at the most promising primers was then tested on fifty unrelated animals. We could identify about 30 primer pairs that amplified a fragment from the dromedary genome with difference in amplicon
size from that of the original species. As expected, most of the working primers were from the Bactrian camel, followed by the new world camelids primers. However, only about thirty percent of the primers showed allelic difference with dromedary genome, out of which 7 were di-nucleotide STRs, and 2 were tetra-nucleotide STRs. These loci have the potential for being included in parentage testing protocols."

Key words: Short Tandem Repeats (STRs), dromedary, Heterologous primers, parentage testing

ДРОМЕДАР ТҮЙЕЛЕРІНІҢ ШЫҒУ ТЕГІН АНЫҚТАУДАҒЫ ҚЫСҚА ТЕРМИНАЛДЫҚ ҚАЙТАЛАУ (ҚТҚ) ГЕТЕРОЛОГИЯЛЫҚ ПРАЙМЕРЛЕРМЕН ГЕНОМҒА СКРИНИГ ЖАСАУ

Түйелердің шығу тегін анықтауда қысқа терминал қайталау (ҚТҚ) пайдаланғаны жайында деректер өте көп болғанымен, танымдық локус жайында зерттеулерді түбегейлі жүргізу қажеттілігі бар. Түйелер жайында белгілі ҚТҚ негізінен тұтығуды қалыптастыратын ди-нуклеотид локустармен белгілі. Егер локустар қатарына тетра-нуклеотид локусы қосылса, шығу тегін анықтауда біраз өзгерістер болар еді. Осы мақсатта 150 гетералогиялық әр түрлі дануарлардан тұратын ҚТҚ праймерлері түйе геномына бақыланды: жануарлар ішінде бактриан, альпака, лама, гуанако, викунья және шошқалар болды. Скрининг екі этапта жүргізілді: бірінші праймер 20 туыс емес жануарлардың панелін қолдану арқылы жасалды. Бұл праймерлерде ди-нуклеотидтер және тетра-нуклеотидтер ҚТҚ болды. Келесі топта туыс емес 50 жануар бақыланды. Нәтежиесінде біз 30 жұп праймер алғашқы түрлерінен түйенің микстурасын қамту мөлшерінде (ампликон) айырмашылық бар екендігі анықталды. Алайда, праймерлер шамамен тек отыз пайыз аллельді айырмашылықты көрсетті, оның ішінде 7 ди-нуклеотидті ҚТҚ және 2 тетра-нуклеотидті ҚТҚ болды. . Бұл локустарды шығу тегін анықтаудағы зерттеулер хаттамасына қосуға болады.

Түйінді сөздер: қысқа терминал қайталау (ҚТҚ), дромедар, гетерологиялық праймерлер, шығу тегін анықтау